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Supplementation of alpha lipoic acid on serum biochemical, minerals and antioxidant status in broiler chicken fed diet with animal fat

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Abstract
The present experiment was conducted to evaluate the effects of supplementation of alpha lipoic acid on serum biochemical parameters, minerals and antioxidant status in broiler chicken fed diet with animal fat. Eighty day old straightrun commercial broiler chicks (Vencobb) were randomly grouped into two treatment groups (T1 and T2) with four replications of ten chicks each and The control group (T1) were prepared with five percent animal fat as per BIS (1992) specifications and T2 were supplemented with 100 mg of alpha lipoic acid/kg of feed containing animal fat. Alpha lipoic acid supplementation did not show any effect on serum glucose, albumin and minerals between treatment groups, whereas, serum protein level was significantly increased. The serum reduced glutathione and superoxide peroxidise level was significantly increased (P<0.01) compared to control group. It can be concluded that, supplementation of alpha lipoic did not affect the serum glucose, albumin and minerals, however improved the serum antioxidant status and protein in broiler chicken fed with diet containing animal fat.

Keywords: Alpha lipoic acid, antioxidant status, broiler chicken, serum biochemical parameters

Introduction
In poultry sector, Maize is the primary energy source for formulating a broiler ration. Now a days, due to more utilization of maize for ethanol production and for human consumption. Maize cost is increasing drastically due to its competition for human consumption and meanwhile availability also reduced for concentrate feed preparation [28]. In order to overcome this problem, an alternate energy source can be utilised for partial replacement for maize. Recently, animal fats are available at competitive prices compared to other energy sources, which can be effectively utilised due to its high energy content and also for its extra calorific effect which improves the consistency and quality of feed [25]. Use of higher levels of animal fat in the feed may increase in fat deposition and oxidative stress in broilers [22], which is most often characterized by elevated lipid peroxidation or reactive oxygen species in blood. This may result in reduced immune potential in birds leading to greater disease susceptibility and suboptimal production. In order to overcome these issues, addition of feed additives is the most practical way to manipulate the fat deposition and to reduce the oxidative stress in broilers. Recent years, Alpha Lipoic acid has gained much interest as a feed additive for altering fat deposition and to combat oxidative stress due to its antioxidant activity [24].
Lipoic acid (LA) also known as thioctic acid (1,2-dithiolane-3-valeric acid) is a naturally occurring compound in microorganisms, plants and animals. Alpha-lipoic acid is both fat and water soluble and therefore, it can be easily absorbed and transported across cell membranes resulting in optimal nutrient availability. Lipoic acid described as metabolic antioxidant, as it is potent scavenger of reactive oxygen species like hydroxy radicals, hypochlorous acid, and singlet oxygen, thereby reduces the oxidative stress in birds [9]. It also acts as an essential cofactor for mitochondrial α-ketoacid dehydrogenase complexes, which is essential for normal oxidative metabolism [32]. Moreover, Lipoic acid and Dihydrolipoic acid (DHLA), the oxidized form of Lipoic acid can effectively remove the free radicals, meanwhile enhances the antioxidants enzymes like CAT, superoxide dismutase and glutathione peroxidise etc. concentrations by providing reducing substrates and regenerating them to reduce free radicals to promote the lipid stability meat products [34]. Therefore, the present study was conducted to evaluate the effects of Alpha lipoic acid on serum biochemical, minerals and antioxidant status in broiler chicken fed with diet with animal fat.

Materials and methods

The present experiment was conducted at Department of Animal Nutrition, College of Veterinary and Animal Sciences, Mannuthy, Kerala using eighty day old broiler chicks, which was randomly allotted to two treatment groups (T_1 and $T_{2)}$ with four replicates of ten chicks each. The control group (T1) were prepared with five percent animal fat as per [8] specifications and T2 were supplemented with 100 mg of alpha lipoic acid/kg of feed containing animal fat. Broiler starter ration was fed to birds up to 4 weeks of age and finisher ration up to 6 weeks of age. Throughout the experimental period, birds were maintained under deep litter system, *ad libitum* feed, drinking water were provided and reared under identical management conditions. The ingredient and chemical composition of dietary treatments of broiler starter and finisher rations were presented in Table 1 and 2.

Table 1: Ingredient composition of broiler starter and finisher ration, %

Ingredients	Broiler starter rations, %		Broiler finisher rations, %	
	T1	T2	T1	T2
Maize	40	40	48.5	48.5
Soybean meal	41.4	41.4	32.89	32.89
Wheat bran	9	9	9	9
Animal fat	5	5	5	5
Dicalcium phosphate	2	2	2.1	2.1
Calcite	1.79	1.79	1.8	1.8
DL-methionine	0.14	0.14	0.04	0.04
Choline chloride	0.1	0.1	0.1	0.1
Trace mineral mixture[1]	0.01	0.01	0.01	0.01
Supplements[2]	0.31	0.31	0.31	0.31
Salt	0.25	0.25	0.25	0.25
Total	100	100	100	100
To 100kg of the above mixture following are added				
Alpha lipoic acid (mg/kg)	-	100	-	100

[1]Trace mineral mixture containing Manganese sulphate-60 g, Zinc sulphate-50 g, Ferrous sulphate-40 g, Iodide-2 g, Copper-5 g, Cobalt-2 g and Selenium-0.3 g. [2]Supplements containing B complex vitamins, Vitamin AB_2D_3K, Toxin binder, Coccidiostat and Liver supplement.

The chemical composition of experimental rations was determined as per the standard procedures [4]. At the end of the experimental period, five birds from each treatment were fasted overnight, slaughtered and dressed as per the standard procedures [7]. Serum Ca, Mg, Cu and Fe concentration were analyzed using Atomic Absorption Spectrophotometer (Perkin Elmer AAS Model 400) while the serum inorganic P (P_i) level was analyzed using blood analyser (Phosphomolybdate method). The serum biochemical parameters like total proteins (Biuret method), albumin (Bromocresol green method) and glucose (GOD-POD method) were analyzed using the kits supplied by Agappe diagnostics, Kerala. The Serum reduced glutathione (GSH) were determined by the method of [20] and Serum superoxide dismutase (SOD) was assayed by the method of [19]. Data collected on various parameters were statistically analysed by the method of [31] using SPSS 16.0 [33]. Means were compared by Independent Samples t Test.

Results and Discussion

The results of serum biochemical parameters are presented in Table.3. It could be seen that the serum glucose (mg/dl) and albumin values (g/dl) recorded in the present study fall within the normal range reported for the species as mentioned by Sastry [27] and Silva [30], respectively.

Table 2: Chemical composition of broiler starter and finisher rations*

Parameters	Broiler starter ration	Broiler finisher ration
Dry matter, %	86.83	87.10
Crude protein, %	23.25	20.14
Ether extract, %	5.48	5.73
Crude fibre, %	4.38	4.16
Nitrogen free extract, %	57.47	62.09
Total ash, %	9.42	7.88
Acid insoluble ash, %	1.90	1.25
Mineral Composition		
Calcium, %	1.41	1.37
Total phosphorus, %	1.23	1.13
Magnesium, %	0.37	0.35
Iron, ppm	90.82	81.80
Copper, ppm	19.92	16.65
Calculated Values		
Metabolisable energy, kcal/kg	2805	2900
Lysine, %	1.27	1.07
Methionine, %	0.34	0.31

* On dry matter basis

The statistical analysis of data indicated that no difference (P>0.05) was noticed between the treatment groups on serum glucose and albumin levels. Similarly, no difference in plasma glucose level was observed in broilers by supplementation of

the α-lipoic acid [12, 13, 14]. Likewise, in Japanese quails, supplementation of lipoic acid did not have any effect on serum albumin level [16]. Supplementation of lipoic acid at 20 mg/kg b.wt rat, did not have any effect on serum albumin level [2].

Table 3: Serum biochemical parameters of birds maintained on two treatments

Parameters	Treatments†		P value
	T_1	T_2	
Total protein (g/dl)	4.16 ± 0.15[a]	4.84 ± 0.16[b]	0.01
Albumin (g/dl)	1.18 ± 0.07	1.34 ± 0.08	0.19
Glucose (mg/dl)	213.29 ± 6.03	226.63 ± 4.19	0.10

a, b -Means bearing different superscripts within the same row differ significantly (P<0.01)

† Mean of five values with SE

Contrary to the present findings, reduction in plasma glucose concentrations was also reported by Hamano [15] in broilers by α-lipoic acid supplementation at 100 and 200 ppm.

The serum total protein levels (g/dl) in experimental birds were also within the normal range for the species as mentioned by [6]. The statistical analysis of the data showed that the α-lipoic acid supplemented group (T_3) had higher (P<0.01) value than that of the control (T_1). In agreement with the present findings, Alegere [3], observed that plasma protein level was increased in lipoic acid injected mice. In contrary to the present findings, Lipoic acid supplementation did not have any effect of total protein level in Japanese quails [16] and in rats [2].

Table 4: Serum Antioxidants status of birds maintained on two dietary treatments

Parameter	Antioxidants level †µg/ml		P value
	T_1	T_2	
GSH	0.11 ± 0.002 [a]	0.13 ± 0.003[b]	0.004
SOD	0.39 ± 0.007[a]	0.44 ± 0.00[b]	0.00

a, b – Means bearing different superscripts within the same row differ significantly (P<0.01)

† Mean of five values with SE.

The serum superoxide dismutase (SOD) and reduced glutathione (GSH) (µg/ml) levels of experimental birds are shown in table 4. The SOD and GSH concentrations were significantly increased (P<0.01) in lipoic acid supplemented group compared to control group. Similar to the results of the present findings, Chen [10] also noticed increase in liver GSH levels of broilers on lipoic acid supplementation at 300, 600 and 900 ppm compared to control group. Arivazhagan [5] also found that GSH level was increased in aged rats injected with lipoic acid at 100/kg body weight. Reduced glutathione concentrations were significantly increased in liver and heart tissue of golden Syrian hamster fed with alpha lipoic acid [35]. Packer [24] suggested that lipoic acid supplementation increased the glutathione level by facilitating the intracellular transport of cysteine, which is required for glutathione synthesis. Whereas, Arivazhagan [5] stated that Lipoic acid increased the affinity for glutathione reductase enzyme, which plays major role in recycling of glutathione and maintenance of GSH concentrations at cellular level. While, Zhang [36] observed that SOD level was significantly increased in broiler chicken, when lipoic acid supplemented at 900 ppm compared to control group. Similarly, Kim [18] also noticed that SOD level was increased in liver and skin by supplementation of lipoic acid in rats. SOD level increased in cortex, hippothalamus and hypothalamus [5] and in retina and brain tissues [1] of rats administered intraperitoneally with lipoic acid at 100mg/kg bwt. Nistico [21] stated that the increase in SOD may be due to the effect of lipoic acid on nerve growth factor (NGF), which provides expression of superoxide dismutase gene which is a factor leading to the increment of SOD.

On contrary, Karaman [17] could not observe any effect on liver reduced glutathione levels by supplementation of lipoic acid in broilers. Halci [11] also observed that reduced glutathione levels in muscle tissue were not affected by lipoic acid supplementation in Japanese quails.

Table 5: Serum mineral concentration of birds maintained on two treatments

Parameters	Treatments		P value
	T1	T2	
Calcium, mg/dl	13.36 ± 0.50	12.67 ± 0.69	0.43
Inorganic phosphorus, mg/dl	4.84 ± 0.07	4.64 ± 0.12	0.18
Magnesium, mg/dl	2.59 ± 0.05	2.60 ± 0.13	0.95
Copper, ppm	0.36 ± 0.04	0.42 ± 0.03	0.23
Iron, ppm	3.19 ± 0.25	3.59 ± 0.19	0.23

† Mean of five values with SE

The Serum mineral concentrations of experimental birds maintained on two dietary treatments are shown in Table 5 and the concentrations were within the normal range for broiler chicken as reported by [30]. The study revealed that there is there is no significant difference (P>0.05) between treatment groups on serum Ca, P, Mg, Cu and iron. In agreement to the present findings, Imik [16] also noticed that lipoic acid supplementation did not have any effect on serum calcium level, however in his study, phosphorous level was increased in Japanese quails. Similarly, Shah [29] also could not find any effect on serum copper level by supplementation of Vitamin – E at 50, 100 and 150µg/kg diet in Japanese quails. In contrast to the present findings, serum calcium and phosphorus level was significantly increased in broilers supplemented with vitamin– E at 250 mg/kg diet [26].

Conclusion

The findings of the present study indicated that supplementation of alpha lipoic acid at 100 mg/kg diet did not have any effect on serum minerals, albumin and glucose level in broiler chicken fed with animal fat containing diet, however increased SOD, GSH and serum protein levels. Thereby, alpha lipoic acid can be used as a feed additive to improve the antioxidant status to improve the quality of meat in broiler chicken.

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